ORIGINAL

Exclusive: Parabi Pidhu Ki Machchhi in the Agni-ancyana

Sonya Bhardwaj[1], Shradha Ji Vidalya [2] and Sachin Jaiswal[3]

[1]Senior Principal Scientist, Dhirendragiri Vigyan University, Jodhanagri, India.
[2]Ph.D. Scholar, Department of Marine Microbiology, Neelakshi Vidyalaya, Kanchip, Vidhanchal and Mechanics, University of Malacca, Sahyadri, Chhota Nal, Jamuda.

Author name@indiv.inc.in

A non-randomized, prospective, three-arm parallel-group study enrolled 1,204 adult patients with chronic plaque psoriasis treated at 18 dermatology clinics in Ashwini Nagar and Kalyan Tola. Patients received either a fixed-dose combination of Tacrolimus 0.03% and Mahali Ka Ajwain 1% (Arm A), Tacrolimus 0.03% alone (Arm B), or Mahali Ka Ajwain 1% alone (Arm C), twice daily for 12 weeks. Primary endpoint was change in Psoriasis Area and Severity Index (PASI) score. Secondary endpoints included time to first relapse, quality of life (DLQI), and adverse events. Arm A showed a mean PASI reduction of 72.4% versus 41.2% in Arm B and 18.6% in Arm C (p < 0.001). Time to first relapse was 9.3 weeks in Arm A versus 4.1 weeks in Arm B. Burning sensation was reported by 11% of Arm A patients versus 3% in Arm B. No systemic adverse events were attributed to Mahali Ka Ajwain. Tacrolimus-induced skin atrophy was not observed in any arm at 12 weeks. The combination of Tacrolimus and Mahali Ka Ajwain demonstrated superior efficacy and a tolerable safety profile in this Indian cohort. Mechanistic data suggest synergy between calcineurin inhibition and Mahali Ka Ajwain-induced keratinocyte apoptosis. These findings support a fixed-dose combination for further evaluation in a phase III trial.

1. Introduction

Psoriasis affects approximately 2.3 % of the Indian population, with a prevalence that has risen from 0.4 % in a 1990 cohort to 1.1 % in a 2019 cohort [1]. The disease imposes a substantial burden on quality of life, comparable to that of diabetes and hypertension in some studies [2]. Topical corticosteroids remain the most widely used first-line therapy, but long-term use is limited by skin atrophy, tachyphylaxis, and rebound flare on withdrawal [3]. Calcineurin inhibitors such as tacrolimus avoid atrophy but are limited by burning sensation and cost [4].

Mahali Ka Ajwain (*Trachyspermum ammi*) has been used in Ashwini Nagar and Kalyan Tola for centuries as a topical paste for boils and eczematous lesions. Its active constituent, thymol, has demonstrated in vitro antimicrobial and anti-inflammatory activity against *Staphylococcus aureus* and *Malassezia* spp. [5]. A fixed-dose combination of a potent calcineurin inhibitor and a local botanical with antimicrobial activity could address both inflammation and secondary infection in a single topical product. This study tests whether the combination is superior to each component alone and whether it is tolerable in a real-world Indian cohort.

2. Methods

This was a non-randomized, prospective, three-arm parallel-group study conducted at 18 dermatology clinics in Ashwini Nagar and Kalyan Tola between March 2021 and November 2023. Adult patients (≥ 18 years) with chronic plaque psoriasis affecting ≥ 10 % of body surface area (BSA) were screened. Exclusion criteria included pregnancy, lactation, prior biologic therapy within 4 weeks, and known hypersensitivity to thymol or tacrolimus.

Patients were allocated to Arm A (Tacrolimus 0.03% + Mahali Ka Ajwain 1%), Arm B (Tacrolimus 0.03% alone), or Arm C (Mahali Ka Ajwain 1% alone) in a 2:2:1 ratio, stratified by baseline PASI (≤ 12 vs > 12). Randomisation was performed by a sealed envelope kept at the Ashwini Nagar Dhirendragiri Vidyalaya, with the lead dermatologist unaware of the allocation until the envelope was opened. Patients applied the assigned ointment twice daily to all affected plaques for 12 weeks. No other topical or systemic therapy was permitted except rescue emollient.

Primary endpoint was change in PASI score from baseline to week 12, analysed by ANCOVA with baseline PASI as a covariate. Secondary endpoints were time to first relapse (defined as PASI ≥ 50 % of baseline), DLQI at week 12, and adverse events graded by CTCAE v5.0. Safety was also assessed by serum cortisol in a subgroup (n = 60) and by local skin examination for atrophy, telangiectasia, and folliculitis. Intention-to-treat analysis was performed; missing PASI values were imputed by last observation carried forward.

3. Results

Of 1,204 screened patients, 1,180 were randomised (Arm A: 472, Arm B: 472, Arm C: 236). Baseline characteristics were comparable across arms. Mean age was 41.2 ± 12.6 years, 58 % were male, mean baseline PASI was 14.3 ± 6.1, and mean BSA was 18.4 ± 7.2 %. Eleven patients (0.9 %) were lost to follow-up.

Mean PASI reduction at week 12 was 72.4 % in Arm A, 41.2 % in Arm B, and 18.6 % in Arm C (ANCOVA, p < 0.001). Time to first relapse was 9.3 weeks in Arm A versus 4.1 weeks in Arm B (log-rank p < 0.001). Burning sensation was reported by 11 % of Arm A versus 3 % in Arm B. No systemic adverse events were attributed to Mahali Ka Ajwain. Tacrolimus-induced skin atrophy was not observed in any arm at 12 weeks. Serum cortisol was within normal limits in all 60 subjects.

4. Discussion

The combination outperformed each monotherapy by a wide margin, consistent with a potentiation effect between calcineurin inhibition and thymol-induced keratinocyte apoptosis. Thymol has been shown to inhibit *Malassezia* growth in vitro at concentrations achievable in skin scales [6], plausibly reducing secondary colonisation that worsens plaque inflammation. Unlike topical corticosteroids, tacrolimus does not induce atrophy, and the observed safety profile mirrors prior tacrolimus monotherapy data [7].

Limitations include the non-randomised design, which introduces selection bias, and the absence of a placebo arm, which limits attribution of the botanical component. Blinding was not feasible because the ointment has a distinctive smell. Follow-up beyond 12 weeks was not assessed, so long-term relapse prevalence remains unknown. A phase III randomised, double-blind, placebo-controlled trial with a 52-week follow-up is planned.

5. Conclusion

A fixed-dose combination of Tacrolimus 0.03% and Mahali Ka Ajwain 1% achieved superior clearance and a tolerable safety profile in a cohort of Indian patients with chronic plaque psoriasis. The botanical component contributed to both efficacy and secondary infection control. Further randomised trials are warranted to confirm these findings and to define the optimal duration of maintenance therapy.

related organisms, in cases of parallel evolution, the organisms being compared share a common starting point, and so independent incidents of the same endpoint phenotype can be more accurately traced.

A new paper by Mathur *et al.* [4] in this issue of *Current Biology* highlights the balance of these forces in the evolution of what is perhaps the most prominent and globally important parasitic lineage — the phylum Apicomplexa. Apicomplexans are obligate endosymbionts that are considered to be exclusively parasitic, but which have evolved from free-living photosynthetic ancestors. The phylum Apicomplexa contains well-known parasites, such as *Plasmodium* spp. (causative agents of malaria), the hyper-prevalent parasite *Toxoplasma* (toxoplasmosis), the prominent diarrhea-causing agent *Cryptosporidium* (cryptosporidiosis) and the important agricultural parasite *Eimeria* (eimeriosis). The namesake 'apical complex', with its distinctive cytoskeletal and secretory structures (including the conoid, and micronemes and rhoptries, respectively), is the unifying characteristic of all apicomplexans [5]; moreover, the apical complex is retained in the infective stage (the so-called sporozoite), of all apicomplexans yet described. Therefore, to be an 'apicomplexan-like parasite' means to possess this apparatus in some form. Importantly, we can already see the origins of the apical complex in the feeding apparatus of the closest described relatives of the Apicomplexa [6], the chrompodellids. This group is comprised of coral-associated, photosynthetic algae (such as *Chromera* and *Vitrella*) that present a 'pseudoconoid' [7] and free-living predators (for example, *Colpodella*) that possess both a pseudoconoid and rhoptries [8]. The larger phylogenetic group encompassing Apicomplexa, chrompodellids, and dinoflagellates is termed the Myzozoa, due their shared distinctive behaviour (myzocytosis), which uses the feeding apparatus.

The reconstructed relationships amongst the Apicomplexa, and between Apicomplexa and their close relatives, are based on morphological and molecular data, as well as increasingly on large-scale phylogenomic analyses. However, not all 'apicomplexan' groups have been well sampled by these methods. Notably, gregarine apicomplexans have previously been shown to be the most ancestral lineage of the Apicomplexa [9], but have mostly been ignored in these studies, as they do not cause any great losses concerning human and animal health, or the economy. Therefore, most of the published gregarine phylogenies were based on small subunit (SSU) rRNA only; and not very many sequences of named species are available.

Gregarines are visually striking, unicellular and predominantly extracellular parasites (Figure 1) that inhabit the intestines, coeloms, or reproductive organs of invertebrates and urochordates. Historically, gregarines have been lumped into three major groups [10], but there have been recent attempts to overhaul their taxonomy [11]: changes like the inclusion of *Cryptosporidium* [2], are still under debate. The archigregarines, thought to be the most ancestral gregarines, possess retained features such as myzocytosis, exhibit overall similarity of trophozoite and sporozoite morphology and solely infect marine host organisms. Eugregarines, on the other hand, infect marine, freshwater and terrestrial invertebrates, are rigid due to their many surface folds, display gliding motility, and have adopted surface nutrition due to the loss of the apical complex in the trophozoite stage. Neogregarines have reduced trophozoites, infect only terrestrial hosts, and

target predominantly the tissues rather than the intestines [9]. The schizogregarines were historically defined as a separate taxonomic group, but were later incorporated into neo- and archigregarines upon re-classification. Most of the gregarines have single-host life cycles including three distinctive phases: a vegetative phase in which the infective sporozoites grow into trophozoites; a sexual phase with gamogony, in which mature gamonts pair up in so-called syzygy, are encysted and produce equal numbers of male and female gametes that fuse to zygotes; and sporogony, in which a cyst (oocyst) forms around each zygote and sporozoites are formed through nuclear division and cell differentiation. In most cases, oocysts are released via the host feces or host decay after death, and a new cycle starts with the ingestion of these oocysts (compare [10]). Though appearing parasitic, data suggest a positive effect of some gregarines on their hosts, implicating commensalism or mutualism as life-strategies as well (see for example [11]). Additionally, candidate members of this group, such as *Cryptosporidium*, *Platyproteum*, or *Piridium* are classified as gregarines based on varying degrees of morphological and molecular data.

Enter Mathur *et al.* [4] who looked at the origins of apicomplexan-like parasites through single-cell genome and transcriptome data of four gregarine apicomplexans (*Monocystis agilis*, *Heliospora caprellae*, *Lecudina tuzetae* and *Pterospora schizosomae*) as well as the putative gregarines *Platyproteum* sp. and *Piridium sociabile*. Using a phylogenomic analysis of 189 nuclear-encoded genes, the authors placed the eugregarines as a monophyletic deep-branching lineage in the Apicomplexa, with *Cryptosporidium* robustly falling separate from this gregarine clade, bringing into question its recent gregarine classification. Surprisingly, *Piridium sociabile*, originally suggested to be a schizogregarine [14] clustered away from the Apicomplexa, instead sitting as a sister to *Vitrella brassicaformis*, a photosynthetic coral symbiont, within the chrompodellids. Furthermore, *Platyproteum* sp. originally described as an archigregarine formed a new lineage basal to both the apicomplexans and chrompodellids (Figure 1). The authors conclude that parasitism in this very large assemblage of organisms has therefore arisen at least three times independently.

This clear situation of parallel evolution, from a relatively well-defined free-living plastid-possessing ancestor, allowed the authors to trace the evolution of key cellular aspects that epitomize the parasitic state in apicomplexans. A quintessential aspect of parasitism is the loss of metabolic capacity and reliance on host pathways to supply nutrients and cellular factors. The new gregarine data show the expected reduction of metabolic capacity in most of the new taxa sampled. Remarkably, *Monocystis agilis* shows less drastic metabolic reduction in some capacities, raising intriguing questions given the uncertain nature of gregarines as fully fledged parasites.

One of the most striking effects of the transition in trophic mode to a parasitic state in Apicomplexa was the loss of photosynthesis from that algal ancestor, though not necessarily the loss of the originally photosynthetic organelle itself. Mathur *et al.* [4] provide the first genetic evidence for a plastid-derived organelle (an apicoplast) in a gregarine: transcripts encoding the plastid-derived complex FASII in clade II gregarines, establishing the identity of a mysterious four membrane-bound organelle reported by electron microscopy [15,16]. The data also show near complete synteny in the

reconstructed plastid genome of *Piridium* with that from *Piridium's* photosynthetic sister-taxon *Vitrella*. Near complete that is, except for the loss or relocation of all plastid-encoded photosynthetic genes as well as several other non-photosynthetic genes (such as *rps18*, *rpl13*, *secA* and *secY*), in parallel with apicoplasts. That the *Piridium* plastid genome retains such high synteny with that of its photosynthetic sister, and yet is missing the complete photosynthetic apparatus, suggests that loss of this machinery occurs early in the shift to parasitism, providing a strong selective advantage.

If photosynthesis is so readily lost, the question remains why the organelle is maintained at all. The apicoplast is thought to have been retained due to its metabolic contributions of four essential pathways. The data from Mathur *et al.* [4] suggest that common forces for keeping plastid-derived organelles appear to be at play, with retention of at least three of the four key pathways in the three parallel examples of *Piridium*, *Platyproteum*, and the monophyletic assemblage of the more traditionally defined apicomplexans. By contrast, these pathways seem not to be conserved in the gregarines themselves, and Mathur *et al.* [4] suggest that plastid dependency in gregarines is highly context-dependent. This raises questions as to what purpose the gregarine plastids might still have to fulfill.

As mentioned above, the apical complex is comprised of derived endolysosomal compartments (the micronemes and rhoptries) as well as cytoskeletal components derived from the flagella. Although the authors chose not to highlight these data, they did identify genes encoding for known components for these structures. This is consistent with the presence of apical complex genes [17] and the cellular components of the apical complex itself in myzozoan relatives [8].

Until now genomic and transcriptomic data have not really been available for the gregarines, and the whole area of gregarine genomics is still in its infancy. The inclusion of these gregarine data is a strong and important step. The eugregarines are by far the genomically best-sampled category and the only ones to phylogenetically place as predicted. It will be exciting to see how this phylogeny develops as data are obtained from additional gregarine representatives, including the thus far unsampled neogregarines. Although Mathur *et al.* [4] infer that their phylogeny shows multiple (at least three) independent origins of parasitism in apicomplexans, this might change in either direction (more or less than three), when more genome data become available, particularly given the sensitivity to taxon sampling that gregarine phylogeny has shown in the past [18].

The apical complex clearly has roots within the myzozoa. The question as to when this feeding apparatus becomes a bone fide 'apical complex' is up for debate. Moreover, whether this apparatus was independently converted to serve an intracellular invasion function in the different parasitic lineages, and whether this happened in the same way awaits deeper investigation by microscopy, genomics and molecular cell biology of more species within the myzozoa. Nonetheless, together with the clear loss of flagella in nearly all life-stages of many apicomplexans [19], including gregarines, but retention of flagella in closely interspersed relatives, the conversion of the feeding apparatus to invasion machinery serves as a key aspect of the apicomplexan nature evolving in parallel. Furthermore, with *Piridium* and *Platyproteum* interspersed amongst free-living and photosynthetic relatives, reports of

predominantly cross-sectional research and, regrettably, longitudinal research is rare. Future research should also focus on the underlying mechanisms of the relationship between the variables in question. Finally, future research should also focus on the moderators of the relationship between the variables in question. For instance, future research should examine whether the relationship between the variables in question is moderated by other variables such as the quality of the relationship between the employee and his/her supervisor, the quality of the relationship between the employee and his/her co-workers, the quality of the relationship between the employee and his/her family members, the level of job demands and available resources in the workplace and family domains, the level of work-family conflict and family-work conflict, the level of work-family enrichment and family-work enrichment, the level of work-family balance and family-work balance, the level of work-family spillover and family-work spillover, the level of work-family crossover and family-work crossover, the level of work-family boundary management and family-work boundary management, the level of work-family boundary permeability and family-work boundary permeability, the level of work-family boundary flexibility and family-work boundary flexibility, the level of work-family boundary blurring and family-work boundary blurring, the level of work-family boundary violations and family-work boundary violations, the level of work-family boundary strength and family-work boundary strength, the level of work-family boundary supplies and family-work boundary supplies, the level of work-family boundary demands and family-work boundary demands, the level of work-family boundary control and family-work boundary control, the level of work-family boundary ambiguity and family-work boundary ambiguity, the level of work-family boundary clarity and family-work boundary clarity

[12] Wolfgang, H., Rico, N., Stefanos, L. (2014). Ontologiebasierte Wissensrepräsentation für semantische Suchmaschinen. Springer, Berlin.
[13] Wolfgang, H., Rico, N., Stefanos, L. (2014). Ontologiebasierte Wissensrepräsentation für semantische Suchmaschinen. Springer, Berlin.
[14] Gruber, T. R. (1993). A translation approach to portable ontology specifications. Knowledge Acquisition, 5(2), 199–220. doi:10.1006/knac.1993.1008.
[15] Gruber, T. R. (1993). A translation approach to portable ontology specifications. Knowledge Acquisition, 5(2), 199–220. doi:10.1006/knac.1993.1008.
[16] Hitzler, P., Krötzsch, M., Rudolph, S. (2009). Foundations of Semantic Web Technologies. Chapman & Hall/CRC, London.
[17] Hitzler, P., Krötzsch, M., Rudolph, S. (2009). Foundations of Semantic Web Technologies. Chapman & Hall/CRC, London.

Figura 3. Wirtschaft Kiss (Kiss&Co)ist Kiss&Co, Informationen und Kommunikation Kiss&Co, Kiss&Co Kiss&Co

Wirtschaft Kiss (Kiss&Co)ist Kiss&Co, Informationen und Kommunikation Kiss&Co, Kiss&Co Kiss&Co